

AMINO ACID REQUIREMENTS OF ORAL LACTOBACILLI

STEWART A. KOSER AND JOAN L. THOMAS

From the Walter G. Zoller Memorial Dental Clinic and the Department of Microbiology, The University of Chicago, Chicago 37, Illinois

Previous communications from this laboratory have dealt with the vitamin requirements of a collection of oral lactobacilli.¹⁻³ As part of a continued investigation of the nutritive requirements of these organisms the amino acid needs also have been studied and the results are presented in this report.

Although the amino acid needs of some lactobacilli, particularly the strains suggested for use in microbiological assays, have been studied in considerable detail, the requirements of oral lactobacilli have been relatively neglected. In a general way, however, it is usually supposed that the amino acid requirements of lactobacilli from the mouth are probably as complex as those of lactobacilli used for assays or those from dairy and fermentation sources. Earlier attempts to cultivate oral lactobacilli in simple synthetic mediums uniformly met with failure. During the studies on vitamin requirements it became apparent that protein hydrolyzates were necessary to supply a number of amino acids and that it was advisable to supplement acid hydrolyzates with tryptophane and cystine. Aside from this recognition of the importance of a number of amino acids for oral lactobacilli, relatively little information of

specific needs has been available; Weisberger and Johnson⁴ noted that tyryptophane was needed by an oral lactobacillus, and Dreizen and associates⁵ determined the needs of an *L. acidophilus* strain.

In contrast, the amino acid needs of lactobacilli from other sources have been studied in detail. Typical of these reports are those dealing with *L. arabinosus* 17-5 (8014) by Kuiken, Norman, Lyman and Hale⁶ and by Hegsted,⁷ with *L. pentosus* 124-2 (8041) by Krueger and Peterson,⁸ and with *L. casei* by Hutchings and Peterson.⁹ The amino acid needs of the foregoing species along with *L. fermenti* 36 (9338) and a number of others (though apparently none of definitely oral origin) were also studied by Dunn and associates.¹⁰ The needs of two strains of *L. leichmannii* were determined by Schweigert, Guthneck, and Scheid.¹¹ A detailed review of the use of various microorganisms—lactic acid producing and others—for amino acid assay was published by Dunn,¹²

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and more recently Hendlin¹³ discussed some of the problems connected with use of lactic acid bacteria in microbiological assays.

It has become clear that the needs of the foregoing lactobacilli are complex; often a number of amino acids are necessary to secure satisfactory growth. When the amino acid needs have been studied by the procedure of omitting singly each amino acid from a mixture of 18 or 20 amino acids, often 8, 10 or more amino acids have been found necessary for growth. However, this procedure has not always yielded the whole story of amino acid needs. Thus, Hegsted⁷ pointed out that although 10 amino acids were determined as essential for *L. arabinosus* 17-5 by this procedure, when the 10 were supplied in the absence of the others the mixture was not adequate for growth. For "good growth" 13 amino acids, out of a total of 19 studied, were needed. Similar findings were reported by Hutchings and Peterson⁹ for *L. casei* and by Krueger and Peterson⁸ for *L. pentosus* 124-2; 16 or 17 amino acids were necessary for satisfactory growth of these lactobacilli.

The amino acids often found essential for the foregoing lactobacilli include valine, isoleucine, leucine, arginine, glutamic acid, cystine or cysteine, tyrosine, phenylalanine, and tryptophane. But for satisfactory growth usually several, or at times all, of the following have been used in addition to the others: alanine, serine, threonine, lysine, aspartic acid, methionine, histidine, and proline. The needs have differed with the different species and with different strains within the species. The matter has been complicated further by erroneous nomenclature of some of the laboratory stock cultures of these organisms.

There is a growing realization that lactobacilli isolated from the oral cavity do not constitute a single species or even a subgroup apart from other lactobacilli, but that most of them possess the characteristics of species encountered elsewhere in nature. Thus, the lactobacilli most commonly encountered by Rogosa and associates¹⁴ in an extensive study of 500 oral isolates were similar or identical with *L. casei*, *L. plantarum*, *L. acidophilus*, *L. arabinosus*, *L. fermenti*, *L. buchneri*, and *L. brevis*. Only relatively few isolates possessed other characteristics or were unidentifiable. Similarly, most of the 200 or more oral lactobacilli studied by Tilden and Svec¹⁵ could be fitted satisfactorily into the foregoing species. Both groups of investigators agreed that *L. casei* was the most frequently encountered of the homofermentative type and *L. fermenti* of the heterofermentative type.

In view of these results it might perhaps be anticipated that the amino acid needs of the oral lactobacilli are much the same as those of other lactobacilli which have been studied in more detail. This, however, is not definitely known, and to gain more information concerning needs of oral lactobacilli this study was undertaken. If the amino acid requirements should be found to be essentially similar to those of other homofermentative and heterofermentative lactobacilli (with due allowance for differences at times seen in individual strains), then this would be added evidence for the merging of the oral lactobacilli with other species now recognized. On the other hand, if the amino acid requirements of oral lactobacilli should differ in general from other

13. Hendlin, D., 1952, Bact. Rev. 16: 241-246.

14. Rogosa, M., Wiseman, R. F., Mitchell, J. A., Disraely, M. N. and Beaman, A. J. 1953, J. Bact. 65: 681-699.

15. Tilden, E. B. and Svec, M. 1952, J. Dental Res. 31: 831-838.

lactobacilli, then the difference might be significant from the standpoint of classification, as well as a contribution to their nutritive requirements.

METHODS

Basal medium.—The basal medium contained 18 amino acids as well as purines and pyrimidines, vitamins, salts, acetate, and glucose in the following amounts per 1000 ml of medium: 400 mg of each of DL-alanine, L-aspartic acid, L-glutamic acid; 200 mg of each of DL-isoleucine, DL-leucine, DL-methionine, DL-phenylalanine, DL-serine, DL-threonine, and DL-valine; 100 mg of each of L-arginine monohydrochloride, glycine, L-histidine, L-lysine dihydrochloride, L-proline, L-tryptophane, and L-tyrosine; 50 mg of cysteine monohydrochloride; 10 mg of each of adenine sulfate, guanine hydrochloride, xanthine, thymine, and uracil; 10 μ g biotin, 1000 μ g nicotinic acid, 500 μ g calcium pantothenate, 100 μ g pteroylglutamic acid, 100 μ g para-aminobenzoic acid, 300 μ g pyridoxal hydrochloride, 200 μ g riboflavin, and when heterofermentative lactobacilli were used 500 μ g thiamine hydrochloride. The inorganic salts were 3 g K_2HPO_4 , 3 g NH_4Cl , 200 mg $MgSO_4 \cdot 7H_2O$, and 10 mg of each of NaCl, $FeSO_4 \cdot 7H_2O$, and $MnCl_2 \cdot 4H_2O$. Also 10 g sodium acetate and 15 g glucose were used.

The medium was adjusted to pH 6.8 before autoclaving and was usually about 6.4 to 6.5 after autoclaving. The ingredients for one liter were dissolved in either 900 or 1000 ml of glass redistilled water, placed in 9 or 10 ml amounts in tubes previously standardized for turbidity determination in the Lumetron. After addition of any sterile solutions just before inoculation the volume per tube was approximately 10 ml.

Thiamine and cysteine were added separately from filtered solutions just before inoculation and after the medium had been tubed and autoclaved. Other compounds were added to the medium directly and autoclaved in the medium. When *L. acidophilus* was used the following compounds were added from previously filtered solutions to each 10 ml tube of medium: Tween 80, 1 mg; ascorbic acid, 3 mg; pantethine, 2 μ g, thiamine hydrochloride, 2 μ g, and vitamin B_{12} , 0.1 μ g.

Cultures.—Thirty-three strains of lactobacilli were used for the tests. This number included 28 oral strains and 5 named species from other sources, American Type Culture Collection or other laboratories, which were used for comparative purposes. Twenty-six of these cultures were

homofermentative, 7 were heterofermentative. Within each of these two categories were representatives of different species or varieties found commonly in the mouth. In a separate study of the characteristics based on the usual fermentation and other tests commonly applied to this group of organisms, the 28 oral strains were classified as follows: 9 were *L. casei*, 4 were considered as lactose-negative *L. casei*, 3 were *L. plantarum*, 4 were *L. acidophilus*, 5 were *L. fermenti*, 1 was *L. brevis* and 2 were not assigned.

Of the 9 *L. casei* cultures 3 failed to ferment rhamnose, the other 6 fermented this sugar. The 4 cultures termed lactose-negative *L. casei* resembled *L. casei* in most characteristics but did not ferment lactose; their characteristics were quite different from those of the lactose-negative *L. delbrueckii* and *L. leichmannii*. Similar *L. casei*-like cultures were noted by Rogosa and associates¹⁴ (their variety *alactosus*) and by Tilden and Svec¹⁵ in their studies of the oral flora. The 5 known species from sources other than the mouth were: *L. casei* 7469, another strain identified as *L. casei*, *L. arabinosus* 17-5 (or ATCC 8014), *L. pentosus*, and *L. fermenti* 9338. *L. arabinosus* and *L. pentosus* are at times referred to as *L. plantarum*.

For inoculation of tests, 0.001 ml, secured by dilution, of 24-hour cultures was used for each tube.

Growth and acid production.—Incubation was at 37 C. Growth response of the lactobacilli was followed by measurements of turbidity in a Lumetron colorimeter with a 650 m μ filter. pH determinations were made also in many instances, using a Leeds and Northrup potentiometer.

RESULTS

In the complete medium of 18 amino acids all lactobacilli except *L. acidophilus* produced heavy final growth and most of them grew readily. The 4 *L. acidophilus* cultures did not produce as luxuriant growth as the others but nevertheless grew sufficiently well so that the effect of omissions of amino acids was readily apparent. With these results for comparison, the essentiality of various amino acids was determined by omitting them singly from the basal medium and noting the growth response of the organisms.

The results of numerous tests are not given in detail, but some typical results

showing the effect of omission of each of two amino acids are given in table 1. Here it is seen that when glutamic acid was omitted, with all other amino acids supplied, all strains failed to grow. Glutamic acid is required for growth. When threonine was the only amino acid omitted all homofermentative lactobacilli, except one strain of *L. acidophilus*, grew as well as in the complete medium. Threonine was not required by these organisms. In contrast, 4 of the 5 strains of *L. fermenti* (heterofermentative) required threonine and the remaining one was stimulated by it. An oral strain of *L. brevis* and a type culture collection strain (9338) of *L. fermenti* also required threonine for growth (not shown in table 1). This difference in requirement for threonine

shown by homofermentative and heterofermentative cultures was one of the few differences between these fermentative subgroups of lactobacilli encountered in the study of amino acid requirements.

In a similar way, the needs of the 28 strains of oral lactobacilli and the 5 strains from other sources were tested by omission of each of the 18 amino acids from the mixture. Thus the amino acid requirements, as determined by these tests, are those which become apparent when all amino acids except one were supplied, some of them supplied presumably in considerable excess over the actual amounts needed. The medium contained all other compounds known to be needed for growth of the strains used. A summary of the amino acid requirements as determined by many hundred individual tests, including repetitions to check results, is given in table 2.

On examination of these results several conclusions seem justified:

1. Irrespective of individual differences, the oral lactobacilli as a whole are rather exacting in their amino acid requirements. Most of them have an absolute requirement for at least 8 or more amino acids. Several additional amino acids, though not an absolute requirement, serve to stimulate growth, in some instances quite markedly.

2. Certain amino acids are required by all or almost all cultures, irrespective of strain or species distinctions: arginine, cysteine (or cystine), glutamic acid, leucine, phenylalanine, tryptophane, tyrosine, and valine. Isoleucine is markedly stimulatory for most cultures and required by some.

3. Within each species the individual strains show some differences, as might be expected. Thus, some oral *L. casei* strains were stimulated by aspartic acid (in the presence of biotin) but others

TABLE 1.—Effect on growth of omission of either glutamic acid or threonine.

Species and strain No.	Complete amino acid medium	Lacking only glutamic acid	Lacking only threonine
	2 or 3 days	7 days	2 or 3 days
<i>L. casei</i>			
3	71*	2*	73*
45	85	0	81
46	79	1	74
1003	69	0	80
Lactose-negative <i>L. casei</i>			
4	81	1	79
1009	79	0	83
<i>L. plantarum</i>			
1019	88	1	87
1020	89	2	90
1023	87	2	87
<i>L. acidophilus</i> †			
1025	36	7	35
1026	48	8	14
			8 to 10 days
<i>L. fermenti</i>			
6	88	0	0
41	77	0	80‡
55	89	0	0
78	88	0	0
1004	83	1	0

* The figures in the table represent comparative growth as shown by turbidity. Turbidity determinations were made with a Lumetron colorimeter using a 650 mμ filter. The readings of percent light transmission were subtracted from 100 to show increasing turbidities by higher figures: 0=clear tube; 65–85=heavy turbidity, near maximal or maximal growth.

† The *L. acidophilus* cultures grew slowly and the readings shown are those obtained at 4 or 5 days, rather than 2 or 3 days as used for the other cultures.

‡ Growth of culture 41 was slow in the absence of threonine, requiring usually 8 to 10 days to attain the growth shown here.

TABLE 2.—Summary of amino acid needs of oral lactobacilli.

	<i>L. casei</i> 9 strains	<i>L. casei</i> lactose negative, 4 strains	<i>L. plantarum</i> 3 strains	<i>L. acidophilus</i> 4 strains	<i>L. fermenti</i> 5 strains
DL-alanine*	O	O	O	S	S†
L-arginine	R	R	S	R	R
L-aspartic acid*	S†	S†	O	R	S†
L-cysteine	R§	R	R, S	R	R§
L-glutamic acid	R	R	R	R	R
Glycine	O, S	O, S	O	O, S	O, S
L-histidine	O	O	O	R	O, R
DL-isoleucine	S	S	O	S†	S, R
DL-leucine	R	R	R	R	R
L-lysine	O†	O	O	R§	O
DL-methionine	O	O	O	S, R, O	O
DL-phenylalanine	R	R	S, R	R	R
L-proline	O	O	O	R	O
DL-serine	S	S	O	R, S, O	O
DL-threonine	O	O	O	R, O	R§
L-tryptophane	R	R	O, S	R	R
L-tyrosine	R	R	O, S, R	R	R
DL-valine	R	R	R	R	R

R = required for growth, O = not required, S = stimulatory. The use of more than one symbol opposite an amino acid signifies the result was about equally divided with respect to the number of cultures tested.

* Tests of alanine and aspartic acid were made in the presence of pyridoxal and biotin. In the absence of either of the vitamins, the respective amino acids are more important.

† One exception, required by one strain.

‡ Aspartic acid stimulated growth of many of the cultures; a few grew equally well without aspartic acid.

§ Stimulatory rather than required by one or two cultures.

|| Stimulated growth of an occasional strain though not required.

were not. A few strains of this species were stimulated by histidine, one required lysine for growth while 8 other strains did not. Similar differences were encountered at times among the individual strains of other species.

4. In spite of individual strain differences, a few species distinctions are apparent in a general way. *L. casei* is slightly more exacting with respect to the amino acids needed for optimal growth than is *L. plantarum*. *L. acidophilus* is the most exacting; indeed, some strains of *L. acidophilus* either require or are stimulated by practically all of the 18 amino acids used in the study.

5. The *L. fermenti* cultures (heterofermentative) exhibit a few distinctions from the commoner homofermentative species such as *L. casei* and *L. plantarum*. *L. fermenti* usually requires threonine and is stimulated by DL-alanine in the presence of pyridoxal, but it is not stimulated by serine, as is *L. casei*. Similar results were secured with *L. fermenti* ATCC 9338 and an *L. brevis* culture of oral origin, which are not shown in table 2. *L. brevis* closely resembles *L. fermenti* in many characteristics.

The dispensability of DL-alanine was tested in the presence of pyridoxal. Under such condition omission of DL-alanine had little or no effect on growth of *L. casei* and *L. plantarum*. In additional tests involving the use of DL-alanine in the absence of pyridoxal (not shown in table 2) it was found that many *L. casei* cultures grew poorly. For these organisms DL-alanine did not substitute satisfactorily for pyridoxal in the amino acid medium used in these tests. These *L. casei* strains may be similar to *L. casei* 7469 studied by Snell and associates,¹⁶⁻¹⁸ which required, in the absence of vitamin B₆, not only D-alanine but certain peptides containing L-alanine, rather than free L-alanine.

Among the amino acids which exerted a stimulatory effect on some cultures was glycine. The effect was not pronounced, but was definite and was seen on repeated tests. This effect may have been due to the formation of glucosyl-

16. Snell, E. E., 1945, J. Biol. Chem. 158: 497-503.
17. Holden, J. T. and Snell, E. E., 1949, J. Biol. Chem. 178: 799-809.
18. Kihara, H. and Snell, E. E., 1952, J. Biol. Chem. 197: 791-800.

glycine on autoclaving the medium.¹⁹

Certain other results not included in table 2 may be mentioned briefly. The two oral strains, previously not assigned definitely to any species, were found to have amino acid needs quite similar to those of *L. casei*, which they somewhat resembled in other respects. *L. casei* ATCC 7469 showed essentially the same amino acid requirements as the oral *L. casei* strains of table 2. Known-type *L. arabinosus* and *L. pentosus* cultures, which are closely allied or identical with *L. plantarum*, exhibited requirements essentially similar to the oral *L. plantarum* cultures of table 2. Thus there seemed to be no real differences between the oral lactobacilli of each species and the known-type lactobacilli from other sources, on the basis of the limited number of cultures of each species included in this study. Additional strains were not included since the purpose of the study was to obtain an over-all picture of requirements of the commoner oral lactobacilli, rather than the study of a larger number of strains of one or a few species.

Effect of nonessential amino acids.—

In the foregoing experiments the absence of certain amino acids, when omitted one at a time, exerted little or no apparent effect upon either the speed or final amount of growth when the remaining 17 amino acids were supplied. These amino acids are usually designated as not required or nonessential. Yet when several amino acids of this category are all omitted at the same time, there is often a definite effect upon growth, even though all essential amino acids (as determined on the basis of single omissions) are supplied. Growth under such conditions is slower and not as luxuriant; at times it may

fail to appear, especially in successive transfers.

In the presence of 10 or 11 amino acids, including all essential ones, only a few *L. casei* and some *L. plantarum* cultures grew well. Growth of the others was delayed or was submaximal in amount, even though sufficient quantities of these amino acids were supplied. Growth of all *L. fermenti* cultures was poor in the presence of 13 amino acids. When the omitted amino acids were supplied, either singly or in groups of 2 or 3, they exerted a cumulative effect in improving growth. Usually, from 13 to 16 amino acids were necessary for maximal growth and acid production of *L. casei*, lactose-negative *L. casei*, the remainder of the *L. plantarum* cultures, and *L. fermenti*. The exact needs varied somewhat with the individual cultures. *L. acidophilus* needed practically all of the 18 amino acids for satisfactory growth; only one strain of this species grew slowly when 14 amino acids were supplied. The results with the oral lactobacilli as a group are similar to those noted earlier for the several strains of lactobacilli suggested for assay purposes.⁷⁻⁹

Amounts of amino acids.—The next phase of the investigation was to determine for some of the amino acids the approximate amounts necessary for full growth and acid production. The tests were made by supplying graded amounts of one amino acid in the usual chemically defined medium containing the customary amounts of each of the other 17 amino acids. Several strains of both homofermentative and heterofermentative subgroups were chosen for the tests.

The result of an experiment with valine is given in table 3. Of the 7 strains of lactobacilli tested in the presence of varying quantities of DL-valine, 5 were homofermentative and 2

19. Rogers, D., and King, T. E. and Cheldelin, V. H. 1953 Proc. Soc. Exper. Biol. & Med. 82: 140-144.

TABLE 3.—Effect of graded amounts of DL-valine on growth and acid production.

DL-valine μg/ml	Turbidity							pH						
	Lactobacilli							Lactobacilli						
	4	45	46	1009	ATCC 7469	1004*	ATCC*	4	45	46	1009	ATCC 7469	1004*	ATCC*
0	1	1	2	3	3	1	1	6.2	6.3	6.2	6.2	6.1	6.3	6.3
1	11	11	11	7	12	1	2	5.2	5.2	5.4	5.5	5.2	6.3	6.3
4	29	27	37	27	32	4	31	4.6	4.6	4.5	4.7	4.4	6.2	5.2
10	48	45	48	46	47	22	42	4.2	4.3	4.2	4.3	4.2	5.5	4.9
20	70	49	65	69	65	53	67	3.9	4.0	3.9	4.0	3.9	4.7	4.4
40	78	75	79	77	78	69	84	3.7	3.8	3.8	3.8	3.7	4.4	4.2
100	84	85	81	86	83	70	87	3.7	3.7	3.7	3.8	3.7	4.5	4.2

Turbidity and pH determinations were made at 3 days at 37 C. Turbidity figures are stated as in table 1.

ATCC 7469 is *L. casei*.

* Heterofermentative cultures, *L. fermenti*.

heterofermentative; 2 ATCC strains were included for comparison with the oral strains. Maximal growth and acid production were attained in the presence of either 40 or 100 μg per ml of DL-valine. An increase in the amount to 200 μg per ml (not shown in the table) did not result in heavier growth or lower pH values. Forty μg per ml supported about the same amount of growth as 100 μg in the case of 3 cultures and near maximal growth of the other 4 cultures. Since the results with 40 μg were so close to those of 100 μg, intermediate amounts were not used. With 20 μg and smaller amounts of valine, growth and acid production were suboptimum. A composite result for all 7 strains may be stated as 40 to 100 μg per ml of DL-valine needed for maximal growth under the conditions of these tests.

In a similar manner tests with graded amounts of other essential amino acids were made. In general the same strains of lactobacilli were used, though in some cases not all 7 of them. Composite results of these tests are listed in table 4. It seemed advisable to state the amounts in rather general terms since results with the different strains often varied somewhat. In each case where a range is given, such as 40 to 100 μg per ml, the smaller figure supported nearly maximal growth and acid production, in many cases only a trifle less than that

given by the higher figure. It is evident that the amounts of different amino acids needed for maximal growth differ considerably. On the one hand, 5 μg per ml of tryptophane satisfied the need of most cultures; at the other extreme, 100 μg or more of L-glutamic acid was needed for maximal growth. The latter result, it may be noted, was secured in the presence of 400 μg per ml of aspartic acid. It must be emphasized that these results apply only to the synthetic medium used. Modifications in the medium might well produce changes

TABLE 4.—Amounts of amino acids needed for maximal growth and acid production.

	Homo-fermentative* μg per ml	Hetero-fermentative* μg per ml
DL-alanine†	100 (1)	100 (4)
DL-alanine		40–100 (3)
DL-valine	40–100 (6)	40–100 (3)
DL-leucine	40 (6)	40 (2)
		40–100 (1)
DL-isoleucine	20– 40 (3)	10 (1)
		20– 40 (2)
DL-threonine		20– 40 (4)
		40–100 (2)
L-arginine hydrochloride		
	20 (2)	20– 40 (1)
	20– 40 (3)	40–100 (1)
L-glutamic acid‡	100 (4)	100 (3)
	100–200 (1)	
L-cysteine·HCL	40–100 (5)	40 (2)
		40–100 (1)
L-tyrosine	10– 20 (4)	10– 20 (3)
DL-phenylalanine	20– 40 (3)	20– 40 (1)
	40 (3)	40 (2)
L-tryptophane	5 (4)	5 (3)
	5– 10 (2)	

The figures in parentheses show the total number of strains tested in each case.

* Homofermentative cultures used for these tests included representative strains of *L. casei*, lactose-negative *L. casei*, and *L. plantarum*. The heterofermentative cultures were *L. fermenti*.

† Pyridoxal was omitted from the basal medium in this case.

‡ In the presence of 400 μg per ml of L-aspartic acid.

leading to a somewhat greater or lesser requirement.

The response to tryptophane was studied by making a sufficient number of readings of turbidity in relation to time to construct growth curves of two cultures in the presence of varying amounts of this amino acid. The result with one of the cultures is shown in figure 1; the result with the other was similar. The amount of L-tryptophane needed is only about 20 to 50 times that of the vitamin nicotinic acid. These results were secured in the presence of 1.0 μg . per ml of nicotinic acid. All of

the lactobacilli which we have tested require both tryptophane and nicotinic acid.

pH.—Throughout the investigation numerous determinations of pH were made along with the turbidity readings, but the results have not been stated in detail. In the complete amino acid medium *L. casei*, lactose-negative *L. casei*, and *L. plantarum*, all of which produced heavy final growth, lowered the pH of the medium from the original 6.4 or 6.5 (after autoclaving) to 3.7 or 3.8. *L. acidophilus*, which did not grow as profusely, lowered the pH

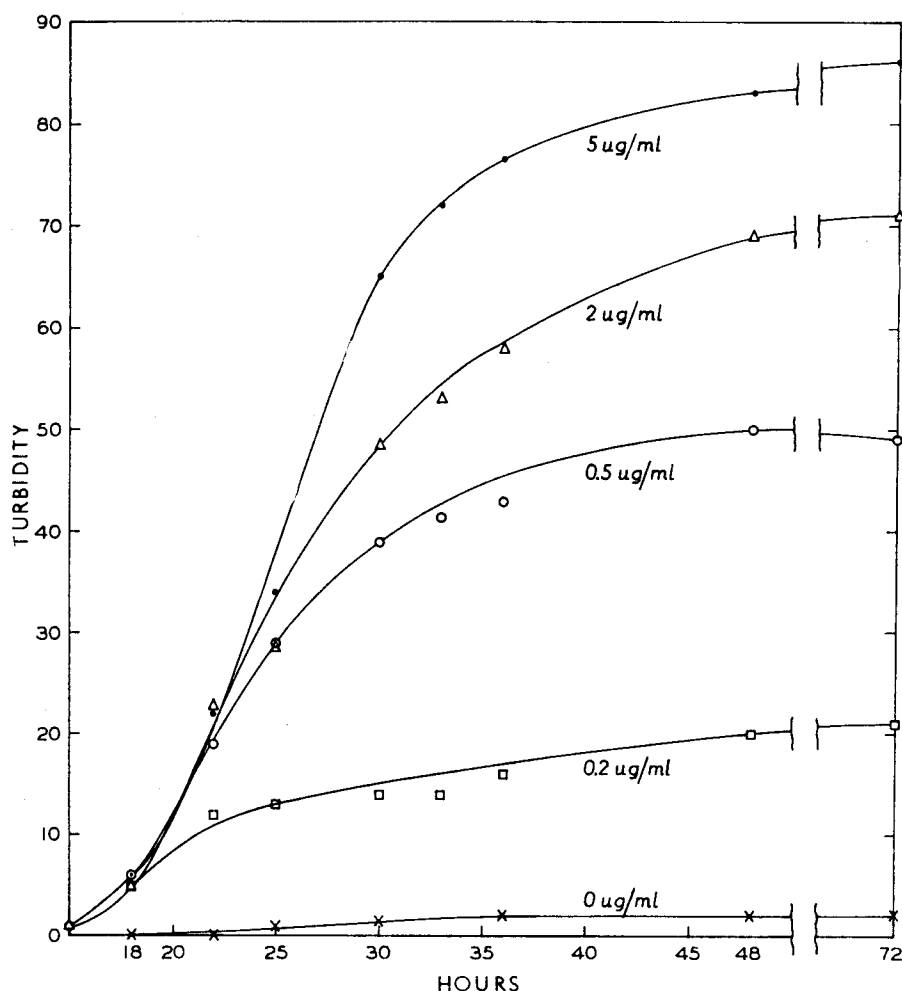


FIG. 1.—Growth response of an oral *L. casei* to different amounts of tryptophane.

usually to 4.1 to 4.6. The heterofermentative *L. fermenti* and *L. brevis* changed the pH to 4.2 to 4.4; turbidity was usually equal to that of *L. casei* and *L. plantarum*.

In those cases where the omission of a single amino acid resulted in no decrease in the amount of growth, the pH values were comparable to those produced by the cultures in the complete medium. Thus, in a medium lacking only lysine the pH values after growth of the lactobacilli were the same as in the complete medium wherever the turbidity was comparable. When the lactobacilli failed to grow or grew only sparsely, as in the absence of glutamic acid (table 1), the pH changed but little from that of the uninoculated medium. Similar results were secured throughout the tests with other amino acids.

In instances where more moderate growth or poor growth occurred, as in the presence of 11 to 13 amino acids, the pH values did not reach as low a point as in the complete medium. Lactobacilli which attained final turbidities of about 30 to 50, for example, reached pH levels of 4.6 to 4.2 for homofermentative cultures and about 5.2 to 4.7 in the case of the heterofermentative cultures. Suboptimum amounts of an essential amino acid also served to limit changes in pH. Some examples have been given in table 3. Throughout the work no instances were observed in which the drop in pH did not bear a general relation to the amount of growth as shown by turbidity.

DISCUSSION

It has become increasingly clear that the nutritive needs of bacteria are influenced by the environment. Consequently the findings with respect to amino acid requirements of lactobacilli relate to specific conditions of growth

and the conclusions are not necessarily valid in other circumstances. Of the various changes which may affect the nutritive needs, it is now recognized that the presence of certain vitamins modifies the amino acid requirement. For example, Lyman and associates²⁰ found that in the presence of vitamin B₆ a strain of *L. arabinosus* did not require threonine, lysine, or alanine; without vitamin B₆ each of these amino acids was essential. The presence of carbon dioxide also affected the amino acid needs. Holden and Snell¹⁷ showed that when vitamin B₆ is not available D-alanine must be supplied for *L. casei*, but when this vitamin is present the organism forms D-alanine. In general, a more complete assortment of amino acids is needed for satisfactory growth when vitamin B₆ is absent. A relationship between amino acids and pteroylglutamic acid has been noted; Harry²¹ found that unrestricted growth of *L. casei* 7469 occurred in mediums without pteroylglutamic acid if certain combinations of L-glutamic acid and DL-alanine were present. That is, in the absence of the vitamin, the two amino acids assumed greater importance. An interrelation between carbon dioxide, temperature of incubation, and the need for phenylalanine, tyrosine, or aspartic acid by *L. arabinosus* was reported by Borek and Waelsch.²²

Antagonistic effects between two or more amino acids also play a part in determining the requirement. Thus, for certain lactobacilli and other lactic acid bacteria threonine exerted an antagonistic effect on the utilization of serine;²³

20. Lyman, C. M., Moseley, O., Wood, S., Butler, B. and Hale, F. 1947, J. Biol. Chem. **167**: 177-187.
21. Harry, E. G. 1951, Biochem. J. **49**: 5-10.
22. Borek, E. and Waelsch, H. 1951, J. Biol. Chem. **190**: 191-196.
23. Meinke, W. W., Holland, B. R. and Sample, M. B. 1948, J. Biol. Chem. **173**: 535-545.

leucine and valine in moderate amounts interfered with the utilization of isoleucine;²⁴ and high concentrations of aspartic acid and ethionine strongly inhibited utilization, respectively, of glutamic acid and methionine.²⁵ In such cases poor growth or no growth results from the use of certain combinations and proportions of amino acids and this antagonistic effect complicates the determination of amino acid requirements.

In presenting the amino acid needs of the lactobacilli studied in this investigation it is emphasized that the results apply only to the conditions used. No attempt has been made in this study to investigate the numerous interacting variables with all of the cultures, though certain relationships became evident which seem worthy of further study. The primary object at this time has been to compare in a general way the needs of a number of oral lactobacilli under a given set of conditions.

A comparison was made of several ATCC or other identified lactobacilli with lactobacilli of oral origin. These results were not included in the tables, but a striking similarity was apparent in the tests. The amino acid requirements of two *L. casei* strains were the same as those of the oral *L. casei* and lactose-negative *L. casei*-like cultures shown in table 2. Also, the needs of *L. plantarum* ATCC 8014 (also known as *L. arabinosus* 17-5) and a previously identified *L. pentosus* strain were almost identical with the oral *L. plantarum*. For all *L. plantarum* cultures arginine was stimulatory rather than a definite requirement, and serine did not stimulate growth as it did in the case of *L.*

casei. *L. plantarum* 8014 did not need cysteine (in the presence of methionine) and *L. pentosus* did not need phenylalanine or tyrosine. The results with *L. fermenti* 9338 and the 5 oral cultures of the same species were similar for the most part; for 9338, arginine and threonine were stimulatory rather than a definite requirement, cysteine was not needed, but proline, in contrast to the 5 oral cultures, was a definite requirement for growth.

A comparison of our findings with previously published results for other lactobacilli can be made in some cases. The amino acid needs of the several lactobacilli used for assay purposes have been studied in detail by several groups of workers. For *L. casei* Hutchings and Peterson⁹ found that arginine, aspartic acid, cystine or cysteine, glutamic acid, leucine, phenylalanine, serine, tryptophane, tyrosine, and valine were essential, while alanine, histidine, isoleucine, lysine, methionine, and threonine stimulated growth. Dunn and associates¹⁰ studied *L. casei* 7469; the amino acids found essential were practically the same as those listed by Hutchings and Peterson,⁹ with the exception that isoleucine was reported as required rather than stimulatory. A comparison of these results with the 9 strains of oral *L. casei* and the 4 lactose-negative *L. casei* given in our table 2 shows that the requirements are very similar indeed.

The amino acid requirements of *L. arabinosus* 17-5 (i.e., *L. plantarum* ATCC 8014) were studied by Hegsted⁷ and by Dunn and associates.¹⁰ The former listed arginine, cystine or cysteine, glutamic acid, isoleucine, leucine, methionine, phenylalanine, tryptophane, tyrosine, and valine as essential; several others, notably aspartic acid, lysine, and threonine were stimulatory. The 3 *L. plantarum* strains of oral origin

24. Brickson, W. L., Henderson, L. M., Solhjell, I. and Elvehjem, C. A. 1948, J. Biol. Chem. **176**: 517-528.
25. Camien, M. N. and Dunn, M. S. 1949, J. Biol. Chem. **179**: 935; 1950, J. Biol. Chem. **184**: 283.

used in the present study are less exacting in certain of their needs, particularly with respect to the methionine requirement and the lack of appreciable stimulation by lysine and threonine (table 2). Costilow and Fabian²⁶ studied the needs of 4 *L. plantarum* strains from cucumber fermentation; cystine, glutamic acid, isoleucine, leucine, threonine, tryptophane, and valine were required by all, while alanine, arginine, phenylalanine, and tyrosine were either stimulatory or essential for one or more of the cultures. Our 3 oral strains differed from the foregoing only in that alanine and threonine were neither required nor appreciably stimulatory. The test for alanine, it will be recalled, was made in the presence of an ample supply of pyridoxal. In spite of a few differences, which may represent largely minor differences between individual strains, there is an over-all similarity in the amino acid needs of the oral cultures, those from cucumber fermentations and from the type culture collection.

The similarity of needs is true also of the *L. fermenti* cultures, though again some of the details differ. Strain 36 (or ATCC 9338) and *L. gordonii* ATCC 8289 (or *L. fermenti* 8289) were studied by Dunn and associates.¹⁰ Arginine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine, tryptophane, and valine were found essential for both of these organisms. The 5 oral *L. fermenti* and one closely-related *L. brevis* of our study, although exhibiting a number of differences between individual strains, did not require methionine in the presence of cysteine. They required cysteine or were stimulated by it.

Only in the case of *L. acidophilus* was there a distinct difference between our

findings and those of others. Little information of the amino acid needs of this species has appeared, probably because this organism is one of the more fastidious of the lactobacilli and it has not been possible until recently to cultivate it in strictly chemically defined media. Also, there has been some confusion regarding the characteristics of this species, or just what should be considered as *L. acidophilus*. Dreizen, Mosny, Gilley, and Spies⁵ found that growth of an oral *L. acidophilus* was completely inhibited when any one of 6 amino acids—cystine, glutamic acid, methionine, phenylalanine, serine, or valine—was omitted, i.e., these amino acids were essential. A number of other amino acids were classed as stimulatory since acid production was markedly reduced in their absence. These were arginine, leucine, isoleucine, proline, tyrosine, or tryptophane. The absence of alanine or threonine also reduced acid production, though not so markedly. The total number of amino acids (14) needed for maximal growth of their culture approached the number needed by our 4 strains. In general, these amino acids were the same, but in our study 12 to 14 amino acids were found to be essential rather than stimulatory, in the sense that their omission resulted in failure to grow.

SUMMARY

1. The amino acid requirements of 28 strains of oral lactobacilli, representing species occurring commonly in the mouth, were determined by omission of each of the amino acids singly from a mixture of 18 amino acids. The requirements are complex; usually at least 8 to 10 amino acids, and often more, are definitely required. Other amino acids stimulate growth, in some instances quite markedly. The results have been summarized in table 2.

26. Costilow, R. N. and Fabian, F. W. 1954, Food Res. 19: 269-271.

2. In spite of some differences in individual strains, a few species distinctions emerge in an over-all way. *L. casei* is slightly more exacting in the amino acids needed for optimal growth than is *L. plantarum*. *L. acidophilus* is more exacting than either *L. casei* or *L. plantarum*. The heterofermentative *L. fermenti*, in contrast to homofermentative *L. casei* and *L. plantarum*, usually requires threonine and is stimulated by DL-alanine in the presence of pyridoxal, but it is not stimulated by serine, as is *L. casei*.

3. Amino acid requirements of lactobacilli from the mouth are quite similar to those of the same species of ATCC or known cultures from other sources. No distinctions were observed which would tend to separate the oral strains from lactobacilli from other sources.

4. Certain amino acids were essential for growth of most lactobacilli, irrespective of species or strain differences: arginine, cysteine (or cystine), glutamic

acid, leucine, phenylalanine, tryptophane, tyrosine, and valine. Isoleucine was markedly stimulatory for all cultures and a definite requirement for some.

5. Amino acids other than those classed as essential on the basis of single omission tests play an important part in growth of lactobacilli. With a supply of 10 to 13 amino acids, including all those determined as essential, growth of *L. planarum*, *L. casei*, and *L. fermenti* is often sparse or slow. For many strains of these species 13 to 16 amino acids must be supplied for prompt and maximal growth.

6. The approximate amounts of each important amino acid necessary for maximal growth and acid production were determined by supplying graded amounts of one amino acid in the presence of an ample supply of the other 17 amino acids. The results differed considerably, from 5 μ g per ml of tryptophane to 100 μ g or more of glutamic acid.